INDUCTION OF CYTOCHROME P4501A IN AFRICAN BROWN HOUSE SNAKE (*LAMPROPHIS FULIGINOSUS*) PRIMARY HEPATOCYTES

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Abstract—Although there have been numerous studies involving fish, birds, and mammals, little is known about the response of the cytochrome P4501A system of snakes to halogenated aromatic hydrocarbons (HAHs). The present study describes the induction of ethoxyresorufin-*O*-deethylase (EROD) in primary hepatocytes of the African brown house snake (*Lamprophis fuliginosus*). Hepatocytes were exposed in multiwell plates to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and four different non-*ortho*-substituted coplanar polychlorinated biphenyls (PCBs 77, 81, 126, and 169). Exposure to TCDD and PCB 126 resulted in a dose-dependent increase in EROD activity, with maximum inducible EROD activities of 177 ± 56 (mean $\pm$ SEM) and 101.1 ± 55 pmol/min/mg protein for TCDD and PCB 126, respectively. None of the other PCBs caused a measurable induction of EROD, which suggests reduced inducibility of snake hepatocytes compared to some vertebrate taxa. Median effective concentrations (EC50s) were 0.16 ± 0.03 nM for TCDD and 8.25 ± 4.14 nM for PCB 126. The relative potency (REP$_{20-80}$) range for PCB 126 was 0.044 to 0.046. Compared to results from in vitro systems using other vertebrate species, both the maximum inducibility and the REPs estimated for *L. fuliginosus* were within the same range as those reported for mammals and the more sensitive bird species but were greater than the values reported for most fish species. In conclusion, induction of EROD activity in primary hepatocytes appears to be a useful approach for evaluating the dioxin-like potencies of aryl hydrocarbon–receptor agonists in snakes. The test system offers a method for rapid screening of reptilian responsiveness to these compounds using smaller numbers of organisms than with in vivo studies, an important consideration for many declining reptile species.

Keywords—Reptile   Aryl hydrocarbon receptor   Relative potencies   Halogenated aromatic hydrocarbons

INTRODUCTION

Induction of cytochrome P4501A mixed-function monooxygenase activity by halogenated aromatic hydrocarbons (HAHs) has been studied in different vertebrate species, including mammals, birds, and fish [1–6]. Halogenated aromatic hydrocarbons are present in almost every environment, and they have been found in tissues from all vertebrate classes, including mammals, birds, fish, amphibians, and reptiles [7,8]. Of the HAHs, dibenzo-*p*-dioxins and coplanar polychlorinated biphenyls (PCBs) have been of specific interest because of their persistence in the environment, their potential to bioaccumulate, and their ability to induce various toxic responses, including dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproduction, development, and the endocrine system [9]. A sensitive response to the binding of HAHs to the aryl hydrocarbon (Ah) receptor is the induction of ethoxyresorufin-*O*-deethylase (EROD). The activity of EROD is directly associated with the induction of hepatic cytochrome P4501A and is commonly used as a marker to test for effects of HAHs in many vertebrate species [2,3,10]. Whereas both the presence and effects of HAHs have been studied intensely in mammals, birds, fish, and turtles [2,3,8,11–14], little information is available on other reptilian species [15,16].

To date, studies evaluating the detoxification capacity of reptiles have been confined to in vivo studies. Taken together, these studies suggest that reptiles generally have less detoxification capacity than do other vertebrates [17–20]. For example, constitutive (i.e., noninduced) cytochrome P450 *O*-dealkylation activities in the American alligator are only 4 to 28% of those measured in rats [19]. Similarly, constitutive hydoxylase activity was least, and *O*-dealkylation activity was second least, in the garter snake compared to a mammal, fish, and frog [21]. Moreover, chemical induction resulted in increased liversomatic index, microsomal protein, and nicotinamide adenine dinucleotide phosphate–cytochrome *c* reductase activity in rats, but not in alligators or snakes [18,19,22]. Induction also resulted in factorial increases in mixed-function oxygenase activity that were two- to fivefold greater in rats and fish compared to alligators [18,19,23]. Because reptiles may play important ecological roles in controlling the flow of nutrients, energy, and contaminants in foodwebs (see, e.g., [24,25]), studies evaluating their sensitivity to contaminants ultimately may be important for their protection and for maintaining the functional integrity of ecological systems. Development of in vitro techniques for examining the sensitivity of reptiles to contaminants, such as primary hepatocyte culture, not only allows for more thorough screening of contaminants but also decreases the number of individuals to be killed, an important consideration for many reptile species that are declining [26]. Successful utilization of primary hepatocyte cultures as a very sensitive marker for HAH toxicity has been demonstrated by several studies with birds, fish, and mammals [2,3,5].

Because a variety of traits make it a suitable study organism, the brown house snake (*Lamprophis fuliginosus*) was selected for the present study. This species is a representative of the family Colubridae, which includes more than 1,800 species and represents 63% of the world's snake diversity [27]. *Lamprophis fuliginosus* is an extremely common, nonvenomous, terrestrial snake found throughout sub-Saharan Africa. The species is primarily nocturnal [28,29] and preys on small mammals and other small vertebrates [30–32]. *Lamprophis fuliginosus* is easily maintained in captivity, with simple husbandry and captive-breeding requirements [33]. In addition, *L. fuliginosus* has been the focus of several recent ecotoxicological studies [33,34].

The objectives of the present study were to establish a primary hepatocyte model to determine the effects of TCDD and selected non-*ortho*-substituted coplanar PCBs on EROD induction in the *L. fuliginosus* as well as to determine the responsiveness of snake hepatocytes to these chemicals and to compare the responsiveness of *L. fuliginosus* to those of other vertebrate species.

MATERIALS AND METHODS

Test animals

In 1998, adult *L. fuliginosus* were acquired from a captive colony at the University of Texas in Tyler, Texas, USA, and a breeding colony was established at the Savannah River Ecology Laboratory in Aiken, South Carolina, USA. Snakes used in the present study represented three generations and spanned one to four years of age. Snakes were housed individually in polyethylene containers ($33.0 \times 18.4 \times 10.5$ cm) with aspen shavings and maintained on a 12:12-h light:dark cycle (photophase starting at 7 AM and scotophase at 7 PM) at 27°C. Snakes were given constant access to water and were fed mice weighing 20 to 40% of snake body mass every two weeks. At the time of the study, two to four snakes were shipped live every two to four weeks to the Michigan State University, East Lansing, Michigan, USA. On arrival at the Michigan State University, snakes were weighed, measured, and killed by decapitation. Snakes were quickly dissected and the livers removed and weighed. Animal care and use protocols were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUF A1999-10024-c2, University of Georgia's Institutional Animal Care and Use Committee, University of Georgia, Athens, GA, USA) and Michigan State University's All-University Committee on Animal Use and Care (AUF 09/03-112-00). The liver somatic index (LSI) was determined as follows:

$$\text{LSI } (\%) = \frac{\text{liver weight}}{\text{body weight}} \cdot 100\%$$

Chemicals and reagents

The four different non-*ortho*-substituted PCB congeners (PCBs 77, 81, 126, and 169) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were obtained from AccuStandard (purity, 99%; New Haven, CT, USA). Working solutions and dilutions were prepared in pesticide residue analysis–grade iso-octane (Burdick & Jackson, Muskegon, MI, USA). The 7-ethoxyresorufin (7-ER) substrate was obtained from Molecular Probes (Eugene, OR, USA).

Preparation and dosing of primary hepatocytes

All hepatocyte preparation and dosing took place under sterile conditions. Following dissection, snake livers were immediately rinsed in phosphate-buffered saline containing 5 mM ethylenediaminetetraacetic acid. The liver was then transferred into a Petri dish containing 10 ml of $1\times$ trypsin (Invitrogen, Carlsbad, CA, USA) and minced using stainless-steel scissors for approximately 2 to 3 min. The trypsin–liver solution was filtered through a piece of cheesecloth, and the trypsin was inactivated by threefold dilution with cell media (Dulbecco's modified Eagle's medium plus Ham's F-12; Sigma, St. Louis, MO, USA). Cells were filtered through a 100-μm nylon screen and centrifuged at 500 *g*. The supernatant was discarded, and the cell pellet was reconstituted in cell media. This procedure (centrifugation and washing of cells) was repeated a second time. Cell density was determined using a hemocytometer and diluted appropriately to a final concentration of 1×10^6 cells/ml in cell media containing 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA), streptomycin (2 U/ml), and penicillin (2 mg/ml). All reagents and solutions were preheated to 30°C before use. Cells were seeded into 24-well (Costar, Bucks, UK) culture plates at 1 ml of cell suspension per well and incubated for 24 h at 30°C in a humidified incubator under 5% CO_2. After this preincubation phase, the medium was changed, and cells were dosed in triplicate with TCDD, PCB 77, PCB 81, PCB 126, and PCB 169 at 10 different concentrations. Concentrations were 0.012, 0.024, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 12.4 nM for TCDD and 0.154, 0.385, 0.77, 1.54, 3.05, 6.1, 12.2, 24.4, 48.8, and 87 nM for PCBs. Final solvent concentration was 1%. A solvent control (carrier solvent, iso-octane) and a blank were run in parallel to each exposure experiment. Exposure duration was 72 h at 30°C. These conditions were determined to result in maximum enzyme induction in a preliminary experiment. Cell condition, including cell morphology (e.g., round, healthy cells vs shriveled, stressed cells) and cell density, was checked under a light microscope on a daily basis.

EROD analysis

At the end of the exposure period, cells were washed three times with phosphate-buffered saline containing Ca^{2+} (0.9 mM) and Mg^{2+} (0.42 mM). The EROD assays were conducted on live cells following a method modified from that described by Kennedy and Jones [35]. Cytochrome P450 activity and protein were measured simultaneously in a microtiter plate using a fluorescent reader (Cytofluor 2350; Millipore, Bedford, MA, USA). Assays were run using 7-ER at 46 μM per well. All assay plates were preincubated for 10 min at 30°C before addition of 7-ER. Time–response curves were developed for each sample to determine optimal reaction time. The EROD assays were run at 30°C over time periods of 3 to 4 h, which were determined to be within the linear range of the time curves. Each experiment was terminated by adding 60 μl of acetonitrile (Burdick & Jackson) containing 0.4 mM fluorescamine (Sigma) per well, and protein concentrations were determined in the same plate following the method described by Kennedy and Jones [35]. Ethoxyresorufin-*O*-deethylase activity was expressed as pmol substrate converted per minute per milligram of protein (pmol/min/mg). Before calculation of enzyme activities, fluorescence measured at the initiation of the experiment (0 h) was subtracted from the final reading to report changes in fluorescence over time.

Data analyses

Enzymatic activities (three replicate wells per individual dose) expressed as pmol/min/mg protein were converted to a
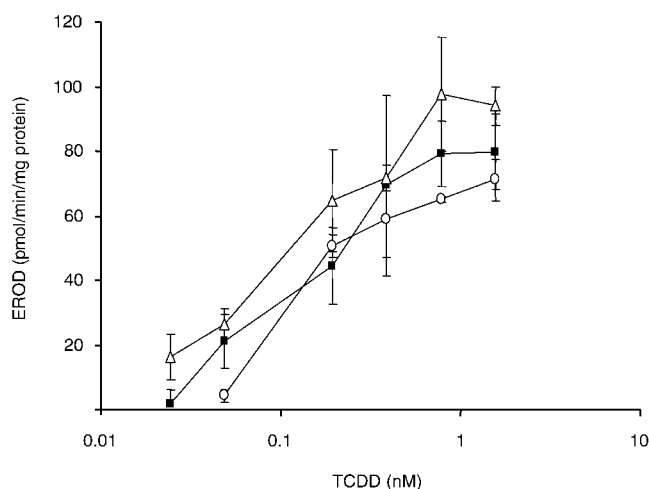
Fig. 1. Dose–response profiles for ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg protein) measured in primary hepatocytes of *Lamprophis fuliginosus* exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Different symbols represent replicate measures that were conducted with hepatocytes derived from one snake. Data are presented as the mean ± standard error of the mean.

percentage of the mean maximum response observed for the TCDD standard curves generated on the same day (%TCDD$_{max}$) to normalize for day-to-day variability and to make responses from different snakes comparable. Solvent controls were subtracted from the absolute activities before this conversion. The linear portions of the dose–response curves were defined by dropping points from the tails until r^2 approximated 0.9, and a linear regression model was fit through the remaining points [36]. At least three data points were used in each case. Effective concentrations (ECs) were calculated based on this model for each individual curve. The same approach was used to establish a linear-regression model for all replicate experiments conducted.

To compare the ECs of test chemicals directly to the TCDD standard, the sample and the standard dose–response must be statistically parallel and have the same efficacy [37,38]. Therefore, multiple point estimates are a more robust estimation of relative potencies (REPs) [36]. Under the assumption that the maximal response of the sample is not less than 20% of the standard maximum, multiple point estimate methods can be used to calculate a REP$_{20-80}$ range to approximate the sample REP as follows [36]:

$$REP_{20-80} \text{ range} = REP_{20} \text{ to } REP_{80}$$

The REP$_{20-80}$ ranges were reported along with conventional REPs based on a single point estimate determined at 50% TCDD/sample maximum to indicate the uncertainty of the estimate [36]. Furthermore, average REP$_{20-80}$ ranges and median ECs (EC50s) generated from dose–response curves from individual snakes were compared to those derived from a general model based on all snakes.

RESULTS

Induction of EROD in primary hepatocytes from L. fuliginosus *exposed to TCDD*

When exposed to TCDD, induction of EROD activity followed a dose–response relationship with increasing enzyme activities at higher doses. One snake out of all the test animals ($n = 17$) was not inducible by TCDD. Results from this snake were omitted when establishing models to calculate ECs and REPs. Initial experiments with *L. fuliginosus* showed that dose–response curves for TCDD were reproducible within the same hepatocyte preparation (Fig. 1) and had similar EC50s (0.19–0.30 nM). Basal and maximum EROD enzyme activities as well as EC50s were found to vary among primary cell cultures prepared from different snakes (Table 1). A Pearson correlation model was applied to identify possible factors, such as sex, age, time since last feeding, body size and weight, and relative liver size (i.e., LSI), that may explain this variability. The only parameters that were significantly and negatively correlated ($r = -0.545$; $p = 0.029$) with each other were maximum inducible EROD activity and age.

To correct for differences in inducibility among cell preparations from different snakes, all further calculations were conducted on data expressed as %TCDD$_{max}$. When average responses measured for each TCDD concentration were fitted into a logistic regression model, a strong and highly significant positive dose–dependent relationship was found between %TCDD$_{max}$ and TCDD (Fig. 2). The EC50s (0.14 nM TCDD) calculated using this model were similar to the average EC50s (0.16 ± 0.03 nM TCDD) from each individual experiment.

Relative potencies of selected PCBs

Of the four coplanar PCBs tested, only PCB 126 caused a measurable induction of EROD enzyme activity in primary hepatocytes of *L. fuliginosus*. Three of the 10 snakes used in the PCB 126 induction experiments were not inducible by PCB

Table 1. Exposure response and basic characteristics of primary *Lamprophis fuliginosus* hepatocytes
for ethoxyresorufin-*O*-deethylase (EROD) induction in individual snakes[a]

	n	Range	Mean ± SEM
Basal activity (pmol/min/mg)	17	26.50–1214.39	261.87 ± 83.60
TCDD			
Max. Ind.[b] (pmol/min/mg)	16	26.14–681.65	177.31 ± 48.66
EC50 (nM TCDD)	16	0.05–0.41	0.16 ± 0.03
PCB 126			
Max. Ind. (pmol/min/mg)	10	NI–385.68	101.1 ± 54.53[c]
EC50 (nM PCB 126)	10	NI–24.13	8.25 ± 4.14[c]

[a] *n* = Number of experimental replicates (snakes); NI = noninducible; Max. Ind. = maximum inducibility; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PCB = polychlorinated biphenyl; SEM = standard error of the mean; EC50 = median effective concentration.
[b] Maximum inducible EROD activity = total EROD activity − basal EROD activity.
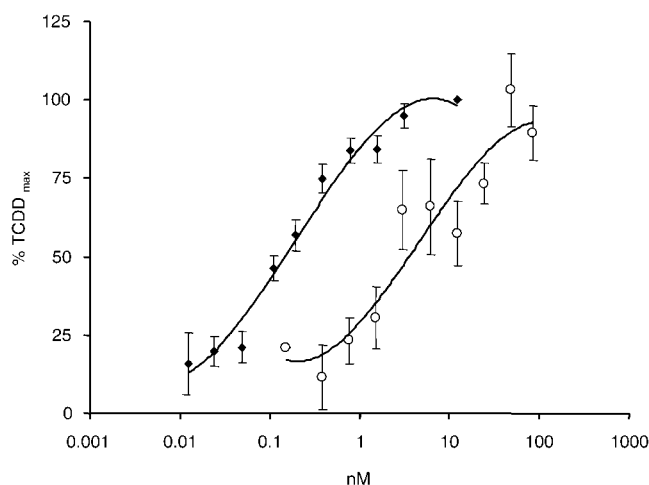[c] Noninducible (NI) snakes ($n = 3$) were omitted for calculation of means.

Fig. 2. Dose–response profiles for ethoxyresorufin-*O*-deethylase activity measured in primary hepatocytes of *Lamprophis fuliginosus* exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; ♦) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126; ○). Data are expressed as the percentage activity of the maximum inducible activity by TCDD (%TCCD$_{max}$) and are presented as the mean ± standard error of the mean.

126 (Table 1). These nonresponsive hepatocyte preparations were omitted in the subsequent analyses. In inducible hepatocytes, EROD activities followed a distinct dose–response pattern parallel to that observed for TCDD (Fig. 2). Efficacy, expressed as %TCDD$_{max}$ for PCB 126, also was similar to that of TCDD. Responses to PCB 126 exposure were more variable than those to TCDD, with a relatively wide range of EC50s measured in the different hepatocyte preparations (Table 1). When comparing relative potencies of PCB 126 to TCDD, the REP$_{20–80}$ range was calculated to be 0.046 to 0.044, with a REP$_{50}$ of 0.045. The EC50s for TCDD and PCB 126, as well as the REPs derived from the present study, were then compared to those determined in primary hepatocytes of other animals, including mammals, birds, and fish (Table 2).

DISCUSSION

Effects of TCDD on primary hepatocytes of L. fuliginosus

The results of the present study demonstrate that TCDD is an inducer of cytochrome P4501A in primary hepatocytes from *L. fuliginosus*. The effects of TCDD could be reproduced in multiple experiments using different snakes, indicating the validity of the established primary hepatocyte model system. Maximum inducibility by TCDD was more variable among individual snakes (26–682 pmol/min/mg protein) than within hepatocyte preparations (99–124 pmol/min/mg protein). One hepatocyte preparation was not responsive to TCDD, which also has been observed occasionally in comparable studies using avian hepatocytes [39,40]. Interestingly, the single unresponsive snake also was the most poor and sporadic feeder, refusing to feed for 120 d before cell preparation. Although periods of digestive quiescence are common in snakes, the observation suggests that the lack of responsiveness of this individual might relate to its physiological condition or metabolic state. Variability in the maximum response also was a function of age, with younger snakes being more inducible than older individuals, as indicated by the significant negative correlation between age and maximum inducibility. Neither sex nor any of the other physiological parameters influenced the inducibility of EROD.

Although results varied considerably among individuals, all hepatocyte preparations followed a similar dose–response pattern, and the EC50 obtained from the overall model was within the margin of error for the average EC50s calculated from individual experiments. This indicates that despite the marked differences in maximum inducibility and background EROD activities among individual snakes, the EC50s of *L. fuliginosus* hepatocytes exposed to TCDD were reproducible. Thus, we conclude that the established model represents a useful and sensitive tool for testing dioxin-like cytochrome P4501A induction potentials in African brown house snakes.

Relative EROD induction potencies of selected planar PCBs

Inducibility of mixed-function oxygenases varies among vertebrate taxa [2,9,22]. Of the four non-*ortho*-substituted co-

Table 2. Comparison of in vitro ethoxyresorufin-*O*-deethylase (EROD) induction potencies (REP$_{50}$) of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and median effective concentrations (EC50s) of TCDD and PCB 126 in *Lamprophis fuliginosus* with EROD induction potencies in primary hepatocytes from other vertebrate species and toxicity equivalence factors (TEFs) given by the World Health Organization (WHO)[a]

	TCDD			PCB126			
	Max. Ind. (pmol/min/mg)	EC50 (nM TCDD)	WHO TEF[b]	%TCDD$_{max}$	EC50 (nM PCB126)	REP$_{50}$	WHO TEF[b]
Reptiles[c]	0.43–11.37	0.045–0.41		83–118	0.019–0.4	0.045	
Fish	1,[d] 2.9[e]	0.13–0.30[e–g] [4, 43, 44]	1	25[d], 43[e]	0.37[e]	0.006–0.007[fg] 0.35[e]	0.005
Birds[hi]	34–400	0.009–2.6	1	60–100	0.052–16	0.06–0.3	0.1
Mammals[j]	110–650	0.02–0.68	1	64–115	0.22–340	0.002–0.2	0.1

[a] Max. Ind. = maximum inducibility; %TCDD$_{max}$ = percentage of the mean maximum response observed for the TCDD standard curves generated on the same day.
[b] Van den Berg et al. [9].
[c] *Lamprophis fuliginosus* (present study).
[d] Smeets et al. [3].
[e] Hahn [44].
[f] Villeneuve et al. [4].
[g] Richter et al. [43].
[h] Kennedy et al. [2].
[i] Kennedy et al. [40].
[j] Zeiger et al. [6].

planar PCBs tested in the present study, only exposure to PCB 126 resulted in measurable induction of EROD activity in primary hepatocytes of *L. fuliginosus* at the concentrations tested. Most fish, as well as some bird species, were less responsive to many of the coplanar PCBs when compared to mammals [9]. Reptilian species, including snakes [22] and alligators [19], were reported both to exhibit lesser basal EROD activities and to be less inducible than the fish, bird, and mammalian species investigated thus far. The fact that PCBs 77, 81, and 169 did not induce EROD activity in primary hepatocytes of *L. fuliginosus* is consistent with these previous observations of low inducibility in reptiles. In birds, interspecies differences in EROD inducibility can be explained, at least in part, by differences in the affinity of the tested chemicals to the Ah receptor [41]. It is reasonable to assume that such differences in Ah-receptor affinity also occur between different taxa or orders and, thus, may explain the differences in inducibility in *L. fuliginosus* when compared to fish, birds, or mammals. The potency of PCB 126 to induce EROD activity in *L. fuliginosus* is in accordance with earlier findings that described PCB 126 as the most potent cytochrome P4501A inducer of the different PCB congeners tested across vertebrate species [2,9,39,42].

Interspecies comparison in sensitivity to EROD induction

The average EC50 determined for TCDD exposure in *L. fuliginosus* hepatocytes is within the range of those reported in vitro for fish [1,4,43], mammals [6], and the more sensitive bird species [2,40] (Table 2). When comparing our present data with these studies, however, it has to be considered that most of the bird and mammalian studies used a different solvent (dimethyl sulfoxide) in their exposure experiments. The choice of solvent can have profound effects on the sensitivity of primary hepatocytes [39]. Dimethyl sulfoxide has been observed to cause a decrease of approximately 10-fold in the EC50s of chicken embryo hepatocytes when compared to iso-octane, the solvent used in the present study [39]. Comparing the EC50s obtained from the experiments with *L. fuliginosus* to those obtained with iso-octane in the study by Sanderson et al. [39] indicates that snake hepatocytes are as sensitive as chicken embryos to TCDD. Nevertheless, because of these solvent-related differences, and assuming that the differences in sensitivity are species independent, it is possible that the EC50s observed in the present study represent an underestimation of the sensitivity of *L. fuliginosus* to TCDD as compared to other species. The effects of different solvents, however, have not been assessed in the snake model used in the present study; therefore, a direct comparison between *L. fuliginosus* and studies of other species in which dimethyl sulfoxide was used as a solvent is not possible.

The fact that snake primary hepatocytes needed to be incubated for a relatively long time (3–4 h) to achieve measurable conversion of substrate to resorufin when compared to assays with primary hepatocytes from other species (e.g., rats, birds, or fish; ~10–30 min) suggests that *L. fuliginosus* has comparatively low absolute cytochrome P4501A concentrations compared to those in these species. Maximum inducibilities in *L. fuliginosus*, however, were similar to those observed for fish [1,3] but approximately an order of magnitude less than those observed during in vitro studies with higher vertebrates [2,6]. Although it has been well established that large differences exist among vertebrate taxa in terms of maximum inducibility by TCDD and dioxin-like chemicals, the

reasons for these differences remain unclear [44]. Molecular genetic approaches, however, suggest that these differences may be caused by phylogenetic differences in the expression or binding characteristics of the Ah receptor [44,45].

Relative potencies determined for PCB 126 were in accordance with those reported from in vitro studies with other vertebrate species (Table 2). The REPs for PCB 126 in snakes also fell within the wide range of toxic equivalency factors (TEFs) from in vivo and in vitro studies by the World Health Organization [9], which represent a conservative estimate of the toxicity of a compound compared to TCDD from available in vivo and in vitro data; snake REPs were half those of mammals and birds and approximately 10-fold greater than the World Health Organization TEF for fish. Thus, we conclude that whereas hepatocytes from *L. fuliginosus* have lower maximum inducibilities compared to birds and mammals, the REPs for dioxin and PCB 126 are similar to the wide range of REPs and TEFs reported for other vertebrates.

Studies of avian responses to HAHs have found that predatory species were less inducible than herbivorous birds. From an ecotoxicological perspective, this would reduce the general risk for birds in higher trophic levels that results from dietary exposure to these pollutants [39,40]. The maximum inducibility of *L. fuliginosus* hepatocytes by TCDD and PCB 126 was comparable to that of the more sensitive herbivorous bird species, such as the common tern (*Sterna hirundo*) or the ring-necked pheasant (*Phasianus colchicus*). Because snakes are carnivores, they are likely at a higher risk for the accumulation of toxic concentrations of dioxin-like HAHs. To date, however, the ecotoxicological data regarding reptiles, especially snakes, is very scarce [16], making the relevance of these findings unclear.

Although we confirmed with the present in vitro study that reptiles appear to have lesser maximum inducibilities compared to those of other vertebrate species [18–20,22], it seems that the snake species studied here was as sensitive (based on EC50s) to TCDD and PCB 126 as mammalian and the more sensitive bird species. These results emphasize the need for incorporation of reptile species in ecotoxicological studies and risk assessments, especially as they represent key components in both aquatic and terrestrial foodwebs [24,34]. The present study demonstrated that in vitro systems can be developed for reptiles with reproducible results. The development of a primary hepatocyte system is useful for screening reptiles using only a small number of animals, which is an important factor considering that many reptilian species are declining [26]. When deemed to be necessary, such screening techniques ultimately can be of considerable value for designing further in vivo tests of HAH toxicity.

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